Gas chromatographic-mass spectrometric analysis of the acetone extract of *Chromolaena odorata* (L.) using ultrasound-assisted

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***Abstract*—This study aimed to investigate, the chemical composition of acetone leaf extracts of Chromolaena odorata (L.) was analysed by gas chromatography-mass spectrometry (GC-MS). A total of 53 chemical compounds were recognized, accounting for 37.36% of the composition of the acetone extracts. The three major components are Germacrene D (5.73%), 5,6,7-trimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one (3.33%) and Phytol (2.90%).**

***Indexed Terms*— Chromolaena odorata, Germacrene D, 5,6,7-trimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one, Phytol, Gas chromatography-mass spectrometry**

I. INTRODUCTION

Plants have always been significantly used as an abundant source of traditional medicines. *Chromolaena odorata* (L.) R.M. King & H. Rob. is native to South and Central America [1]. *C. odorata* belongs to the Asteraceae familyis. The plant is found(endemic) in the tropical and subtropical species of perennial shrubs [2]. Literature reviews show that the *C. odorata* contained appreciable amounts of bioactive compounds with important therapeutic effects on some pathological diseases [3]. The distinct biological activities of *C.* od*orata* reports on the antibacterial, anticancer, anticonvulsant, antidiabetic, antidiarrheal, antifungal, anti-inflammatory, antioxidant, antiparasitic, hemostatic, wound healing, and hepatoprotective activities [4].

Currently, ultrasound-assisted extractions (UAE) has been reported for the extraction of several botanicals. UAE techniques can accelerate mass transfer from the solid to the liquid phase and increase the yield of extracted phytochemicals [5]. UAE produces cavitation of small bubbles in the solvent via the passage of ultrasonic waves, allowing greater penetration of the solvent into the material and thus increasing the surface area [6]. The UAE method has been shown to accelerate the extraction process, reduce energy consumption and increase the extraction of phytochemicals from plants.

Furthermore, solvent extraction of *C. odorata* leaves is the most widely used method. The extraction of phytochemicals from *C. odorata* leaves is influenced by solvent system. Atindehou et al. tested cyclohexane, dichloromethane, ethyl acetate and butanol solvent type extracts of *C. odorata* leaves [7]. The use of ethanol, methanol, chloroform and a mixture of methanol : chloroform : water in the ratio 12:5:3 for the extraction of *C. odorata* leaves was investigated by Raman et al. [8]. Anyasor et al. tested ethanolic and aqueous solvent system extracts of *C. odorata* leaves [9]. Vijayaraghavan et al. investigated the effects of solvent type (ethanol, methanol, acetone and water) on the extraction of the *C. odorata* leaves [10]. Although the literature on solvent extraction of *C. odorata* leaves has been published by some authors, there is no literature was reported on the acetone solvent extraction on the survey of bioactive components from *C. odorata*.

Gas Chromatography-Mass Spectroscopy (GC-MS) has been widely used to identify the chemical compounds of the secondary metabolites of the plant extract for both quantification and verification purposes. To the best of our knowledge, the chemical compositions of the acetone leaf extract of *C. odorata* has not yet been reported. The aim of the present study

is to investigate the profile of the phytochemical compounds of the acetone leaf extracts of *Chromolaena odorata* (L.) using ultrasound-assisted and identified the bioactive components using gas chromatography-mass spectrometry (GC-MS).

II. MATERIALS AND METHODS

A. Plant and Chemical Materials

The leaves of *Chromolaena odorata* L. (Asteraceae) were collected from the campus of I-Shou University. Acetone and 95% ethyl alcohol were purchased from Macron Fine Chemicals. All reagents were used as received without any further purification.

B. Extraction of Chromolaena odorata Leaves

Freshly harvested *Chromolaena odorata* leaves were completely washed with water and dried for 20 min at 60 °C in an oven. The dried leaves were pulverized into powder with a mechanical grinder. A total of 50 g *C. odorata* dried powder was mixed with 400 mL acetone. The mixture was sonicated at 40 ℃ by an ultrasonic extractor (DC200, DELTA, Kaohsiung, Taiwan) for 1 hour. The extract solution was filtered under gravity with Whatman No.1 filter paper to remove insoluble debris. After filtration, the acetone extracts were concentrated under vacuum at 40 ℃ using a rotary evaporator and obtained 1.680g light green gum. The dried extract was stored at 4 ℃ until analysis.

C. Analysis of phytochemical composition of the acetone extract

The components of the acetone leaf extract of *C. odorata* were performed using the GCMS-QP2020 NX (Shimadzu Corporation, Kyoto, Japan), which has SH-Rxi-5Sil MS gas chromatograph column (30 m length x 0.25 mm ID x 0.25 μm film thickness) coupled to a quadrupole mass spectrometer (MS Detector). The injector temperature was set at 250 °C. The interface and ion source temperatures were set at 280 °C and 200˚C, respectively. The carrier gas was helium (99.99 %) with a constant flow rate of 0.97 mL/min. Spectra were obtained over a scanning range of 35 to 500 m/z. The oven temperature of the GC program was proceeded to increase from 80˚C to 280˚C at a rate of 7 ℃/min and kept at 280°C for 10 min. The extract (0.10 g) was dissolved in 1 mL of 95% ethyl alcohol and vortex-mixed for 3 min, then centrifuged at 15000 rpm for 10 min. The 0.5 μL of extract solution was injected with an auto-injector using the split mode with a ratio of 1:39. The molecular fragments of the phytochemicals of the mass spectra were compared using the database of the National Institute Standard and Technology (NIST 17.0 Mass Spectral Library database, Gaithersburg, MD, USA) and the relative percentages of the chemical constituents were calculated according to the absorption peak areas of the gas chromatograms.

III. RESULTS

Figure 1 reveals the total ion chromatograms (TIC) of each component of the acetone leaf extract of *C. odorata*. The components were identified by comparing their mass spectra against the NIST 17.0 Mass Spectral Library database with matching greater than or equal to 90 %. A detailed analyses in Table 1, 53 different components were recognized from the acetone extracts, accounting for 37.36 % of the total area. Among the 53 different compounds, three major volatile oil constituents (Figure 2) of the acetone extract were Germacrene D (5.73%), 5,6,7-trimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one (3.33%) and Phytol (2.90%) and with moderate amounts of Neophytadiene (1.92%), 5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one (1.63%), [1S-(1.alpha.,4a.beta.,8a.alpha.)]-1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene (1.61%), (Z)-9-Octadecenamide (1.46%), Squalene (1.45 %), 4,11,11-trimethyl-8-methylene-Bicyclo[7.2.0]undec-4-ene (1.40%), Hesperetin (1.36%), [1R-(1.alpha.,3.alpha.,4.beta.)]-4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-Cyclohexanemethanol (1.35%), beta.-Amyrin (1.16%), dl-.alpha.-Tocopherol (1.12%), 1-Hexacosanol (1.11%) and Pectolinaringenin (1.05%). The other components constituted less than 1 % of the total yield of volatile oil compounds.

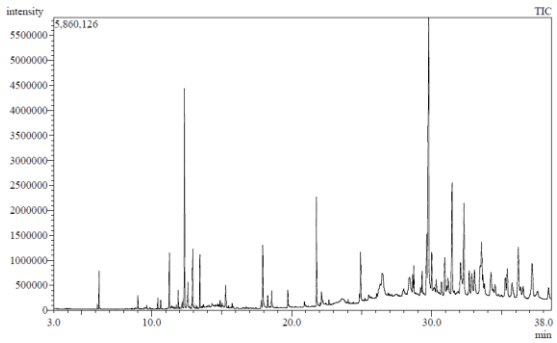


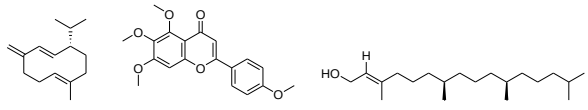
Figure 1: GC-MS total ion chromatograms (TIC) of chemical constituents of the acetone leaf extracts of *Chromolaena odorata*

Table 1: Chemical constituents of *Chromolaena odorata* acetone extract

Compd no.	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	% Area
1	6.12	Geijerene	$C_{12}H_{18}$	162.27	0.1
2	6.24	Geijerene	$C_{12}H_{18}$	162.27	0.84
3	9.01	Geijerene	$C_{12}H_{18}$	162.27	0.33
4	9.66	(3R-trans)-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-Cyclohexene	$C_{15}H_{24}$	204.35	0.07
5	9.88	.alpha.-Cubebene	$C_{15}H_{24}$	204.35	0.04
6	10.44	Copaene	$C_{15}H_{24}$	204.35	0.24
7	10.64	[1S-(1.alpha.,2.beta.,4.beta.)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-Cyclohexane	$C_{15}H_{24}$	204.35	0.21
8	11.26	4,11,11-trimethyl-8-methylene-Bicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$	204.35	1.4
9	11.42	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylene-tricyclo[4.4.0.02,7]decane	$C_{15}H_{24}$	204.35	0.1
10	11.76	(1S,4S,4aS)-1-Isopropyl-4,7-dimethyl-1,2,3,4,4a,5-hexahydronaphthalene	$C_{15}H_{24}$	204.35	0.09
11	11.89	Z,Z,Z-1,5,9,9-tetramethyl-1,4,7,-Cycloundecatriene	$C_{15}H_{24}$	204.35	0.46
12	11.99	(1.alpha.,4a.beta.,8a.alpha.)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-Naphthalene	$C_{15}H_{24}$	204.35	0.05
13	12.19	.gamma.-Muurolene	$C_{15}H_{24}$	204.35	0.18
14	12.34	Germacrene D	$C_{15}H_{24}$	204.35	5.73
15	12.87	(1.alpha.,4a.beta.,8a.alpha.)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-Naphthalene	$C_{15}H_{24}$	204.35	0.13
16	12.93	[1S-(1.alpha.,4a.beta.,8a.alpha.)]-1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene	$C_{15}H_{24}$	204.35	1.61
17	13.03	Zonarene	$C_{15}H_{24}$	204.35	0.07
18	13.19	1,2,3,4,4a,7-hexahydro-1,6-	$C_{15}H_{24}$	204.35	0.07

		dimethyl-4-(1-methylethyl)-Naphthalene			
19	13.26	[1S-(1.alpha.,4a.beta.,8a.alpha.)]-1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene	$C_{15}H_{24}$	204.35	0.02
20	13.44	[1R-(1.alpha.,3.alpha.,4.beta.)]-4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-Cyclohexanemethanol	$C_{15}H_{26}O$	222.37	1.35
21	13.70	(E,E)-1,5-dimethyl-8-(1-methylethylidene)-1,5-Cyclodecadiene	$C_{15}H_{24}$	204.35	0.06
22	14.31	[1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-decahydro-1,1,4,7-tetramethyl-1H-Cycloprop[e]azulen-4-ol	$C_{15}H_{26}O$	222.37	0.13
23	14.55	(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7a-dimethyloctahydro-1H-1,4-methanoinden-8-ol	$C_{15}H_{26}O$	222.37	0.04
24	14.81	(1S,4S,4aS,8aR)-1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-4a(2H)-Naphthalenol	$C_{15}H_{26}O$	222.37	0.07
25	14.89	(2R-cis)-1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-2-Naphthalenemethanol	$C_{15}H_{26}O$	222.37	0.21
26	15.03	.tau.-Cadinol	$C_{15}H_{26}O$	222.37	0.14
27	15.29	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol	$C_{15}H_{26}O$	222.37	0.83
28	15.75	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	$C_{15}H_{24}O$	220.35	0.14
29	17.94	Neophytadiene	$C_{20}H_{38}$	278.52	1.92
30	18.30	Neophytadiene	$C_{20}H_{38}$	278.52	0.34
31	18.57	Neophytadiene	$C_{20}H_{38}$	278.52	0.5
32	19.72	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	0.54
33	21.77	Phytol	$C_{20}H_{40}O$	296.53	2.9
34	22.05	(Z,Z)-9,12-Octadeca-dienoic acid	$C_{18}H_{32}O_2$	280.45	0.08
35	22.14	(Z,Z,Z)-9,12,15-Octa-decatrienoic acid	$C_{18}H_{30}O_2$	278.43	0.56
36	22.66	Hexadecanamide	$C_{16}H_{33}NO$	255.44	0.12
37	23.97	2-dimethylaminoethyl-3-Cyclopentylpropionate	$C_{12}H_{23}NO_2$	213.32	0.03
38	24.04	1-Heptacosanol	$C_{27}H_{56}O$	396.74	0.1

39	24.92	(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	281.48	1.46
40	25.23	Hexadecanamide	$C_{16}H_{33}NO$	255.44	0.07
41	26.07	2-dimethylaminoethyl-3-Cyclopentylpropionate	$C_{12}H_{23}NO_2$	213.32	0.13
42	26.32	.beta.-Amyrin	$C_{30}H_{50}O$	426.73	1.16
43	26.95	Triphenylphosphine oxide	$C_{18}H_{15}OP$	278.28	0.05
44	28.65	1-Hexacosanol	$C_{26}H_{54}O$	382.71	0.59
45	29.43	2-chloro-6-fluorophenyl-4-methoxybenzyl Succinate	$C_{18}H_{16}ClFO_5$	366.77	0.13
46	30.00	Squalene	$C_{30}H_{50}$	410.72	1.45
47	31.17	1-Hexacosanol	$C_{26}H_{54}O$	382.71	1.11
48	32.69	Pectolinaringenin	$C_{17}H_{14}O_6$	314.29	1.05
49	32.87	Hesperetin	$C_{16}H_{14}O_6$	302.28	1.36
50	33.67	.delta.- O-methyl-Tocopherol,	$C_{28}H_{48}O_2$	416.68	0.92
51	35.27	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.71	1.12
52	35.40	5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one	$C_{18}H_{16}O_6$	328.32	1.63
53	36.19	5,6,7-trimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one	$C_{19}H_{18}O_6$	342.34	3.33



a) Germacrene D b) 5,6,7-trimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one c) Phytol

Figure 2: Three major components of the acetone extract from the leaves of *Chromolaena odorata*

IV. DISCUSSION

Phytochemicals are chemical compounds produced by the plants of herbal medicines and have a wide range of bioactive compounds. In this study, the previously phytochemical screening of the acetone extracts of the leaves of *C. odorata* shows that the plant possesses moderate amounts of bioactive substances.

Germacrene D belongs to a class of volatile organic hydrocarbons, the monocyclic sesquiterpene. It was recognized at a retention time of 12.34 minutes and the base peak value of 161.20. Germacrene D is a chiral compound and can serve as an important precursor of many other sesquiterpenes, including cadinene and amorphene [11, 12]. Previous studies have reported that the biosynthesis of the sesquiterpene germacrene D is predominantly via the methylerythritol phosphate pathway for the plant of *S. Canadensis* [13]. In addition to, Germacrene D possessed scavenging activity towards the ABTS radical (IC50 values of 14.5 μg/ml) and antiproliferative effects on tumour cell lines [14].

5,6,7-trimethoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one is a methoxyflavanone compound also called 4',5,6,7-Tetramethoxyflavanone or Scutellarein tetramethyl ether. This compound was identified at a retention time of 36.19 minutes and the base peak value of 327.15. Studies reported that scutellarein tetramethyl ether was extracted from the leaves of *C. odorata* and showed anti-inflammatory activity via the NF-κB pathway [15].

Phytol was identified at a retention time of 21.77 minutes and the base peak value of 71.10. Phytol is an acyclic monounsaturated diterpene alcohol and presents in the composition of some aromatic plants. Reserch data reported that phytol may be useful against mycobacteria and as an antispasmodic, anticonvulsant and Anti-Inflammatory Activity.

CONCLUSION

This study investigates the effects of acetone solvents on the extraction of bioactive compounds from *Chromolaena odorata* (L.) R.M. King & H. Rob. The analysis by GC-MS of the acetone leaf extract of *C. odorata* identified of 53 components. Leaves from *C. odorata* (L.) produced a terpene-rich essential oil. It was found that the essential oils contained sesquiterpenes and diterpenes with percentages of 13.44% and 2.76% in the leaves, respectively. Among the 53 different compounds, the major volatile oil constituents was Germacrene D. The natural compound could be beneficial for use as a natural antioxidant to reduce reactive oxygen species (ROS) in healthcare or to expand their use in cosmetics, food and pharmaceuticals products.

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